By Express Mail
No. EV011854570US

What is claim d is:

1. A method for classifying and counting nucleated bone marrow cells comprising the steps of:

(1) mixing a sample of bone marrow fluid with an erythrocyte lysing agent to lyse erythrocytes in the sample and render leukocytic cells and erythroid cells in the sample suitable for staining, and staining the sample with a fluorescent dye for producing a difference in intensity of fluorescence between the leukocytic cells and the erythroid cells;

(2) introducing the resulting sample to a flow cytometer to detect at least one kind of scattered light and at least one kind of fluorescence;

(3) classifying and counting nucleated bone marrow cells, the leukocytic cells and the erythroid cells with use of a difference in the intensity of the fluorescence and the scattered light;

(4) calculating the ratio of the nucleated bone marrow cells to the erythroid cells or leukocytic cells from the obtained erythroid cell count or leukocytic cell count and the obtained nucleated bone marrow cell count; and

(5) calculating the ratio of the leukocytic cells to the erythroid cells from the erythroid cell count and the leukocytic cell count.

2. The method according to claim 1, further comprising the steps of:

(6) classifying erythroid cells into at least two groups according to maturity of each of the erythroid cells and counting cells in each of the groups with use of the difference in the intensity of the

fluorescence and the scattered light; and

(7) calculating the ratio of erythroid cells in each of the groups to all the erythroid cells from the obtained erythroid cell count in each of the groups and the total erythroid cell count.

3. The method according claim 1 or 2, further comprising the steps of:

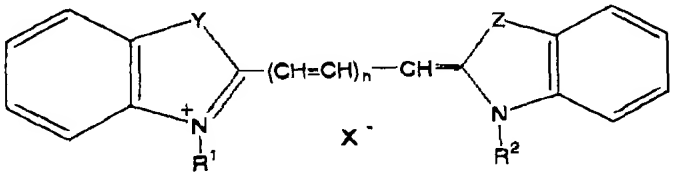
(8) calculating a myeloid cell count by deducting a lymphocyte count and a monocyte count from the leukocytic cell count; and

(9) calculating the ratio of the erythroid cells to myeloid cells from the obtained myeloid cell count and erythroid cell count.

4. The method according to claim 1, wherein the erythrocyte lysing agent is an aqueous solution having an osmotic pressure of 100 mOsm/kg or less and a pH of 2.0 to 5.0.

5. The method according to claim 1, wherein the fluorescent dye comprises one or more dyes selected from the group consisting of:

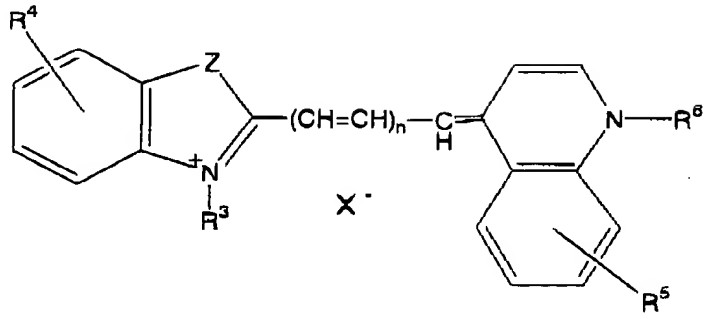
- compounds of formula (1)



(1)

wherein R^1 and R^2 are, the same or different, a hydrogen atom, or an alkyl or alkenyl group optionally substituted by a hydroxyl group; Y and X are, the same or different, a hetero atom or a carbon atom substituted by a lower alkyl group; n is 0, 1 or 2; and X^- is an anion,

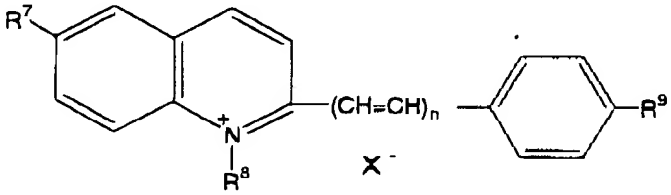
- compounds of formula (2)



(2)

wherein R^3 is a hydrogen atom or an alkyl group; R^4 and R^5 are, the same or different, a hydrogen atom, a lower alkyl group or a lower alkoxy group; R^6 is a hydrogen atom, an acyl group or an alkyl group; Z is a hetero atom or a carbon atom substituted by a lower alkyl group; n is 0, 1 or 2; and X^- is an anion,

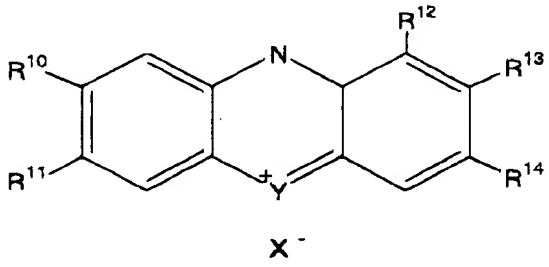
- compounds of formula (3)



(3)

wherein R^7 is a hydrogen atom or a dimethylamino group; R^8 is an alkyl group; R^9 is a hydrogen group or a dimethylamino group; n is 1 or 2; and X^- is an anion,

- compounds of formula (4)

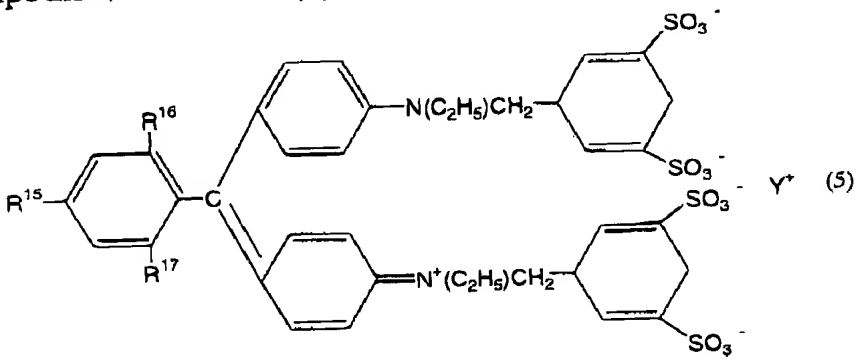


(4)

wherein R^{10} is a hydrogen atom or an alkyl group; R^{11} is a

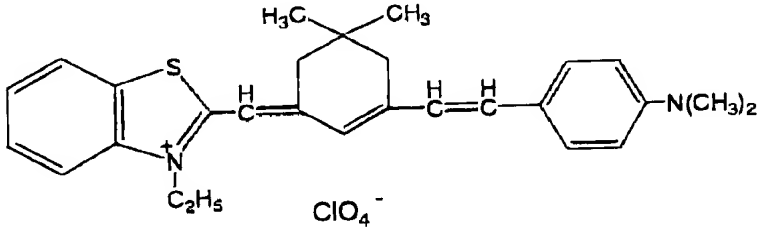
dimethylamino group; R^{12} is a hydrogen atom or an amino group; R^{13} is a hydrogen atom, an alkyl group or an amino group; R^{14} is a hydrogen atom or a dimethylamino group; X^- is an anion; and Y is a hetero atom,

- compounds of formula (5)

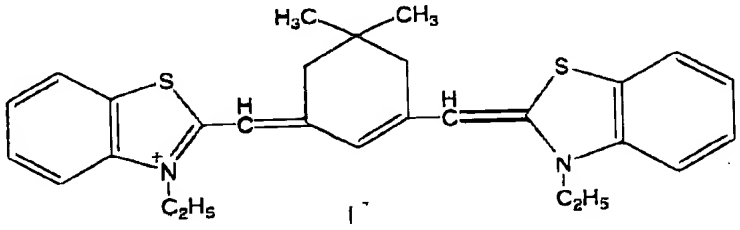


wherein R^{15} is a hydrogen atom or a hydroxyl group; R^{16} is a hydrogen atom or a sulfonic group; R^{17} is a hydrogen atom or a sulfonic group; and Y is a cation,

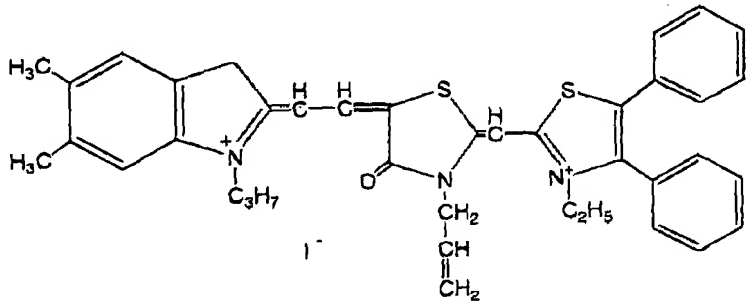
-NK-2825:



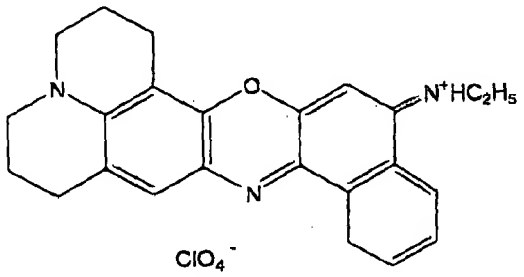
-NK-1836:



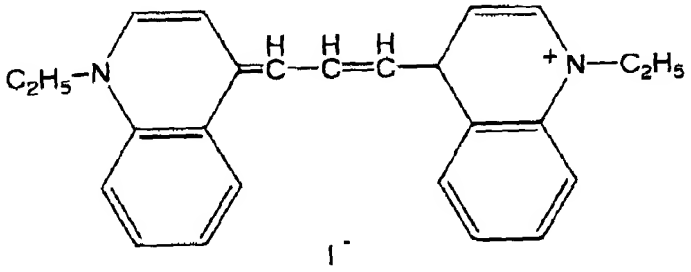
-NK-1954:



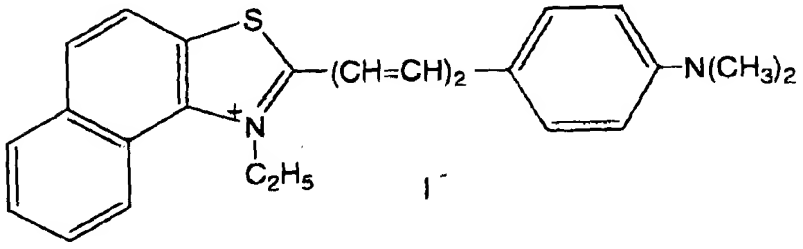
-Oxazine750:



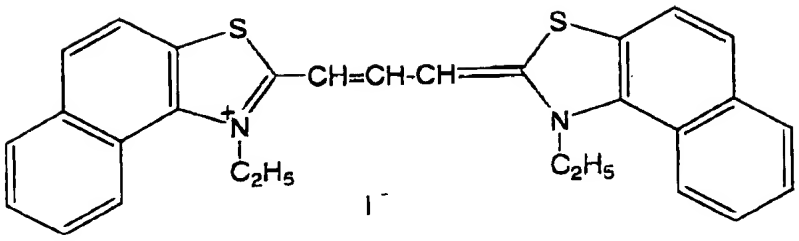
-Cryptocyanine:



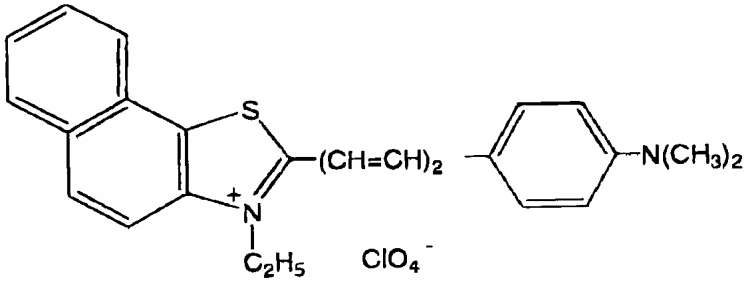
-NK-376:



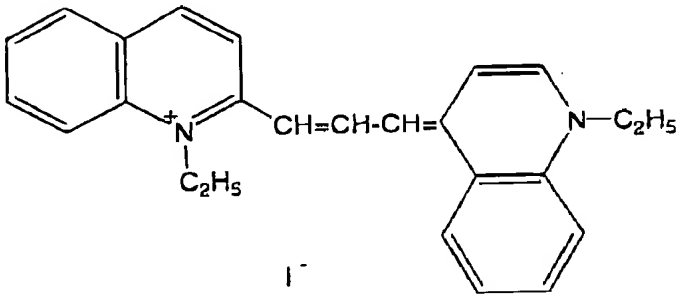
-NK-382:



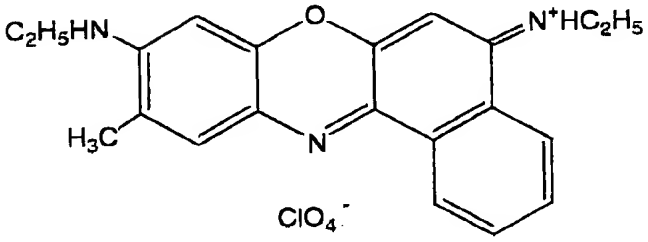
-NK-2711:



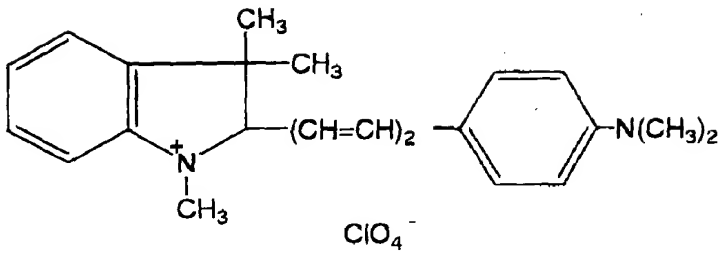
-NK-138:



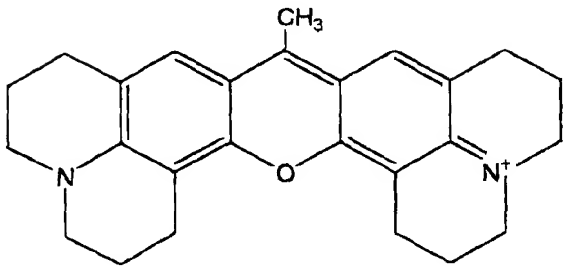
-Oxazine720:



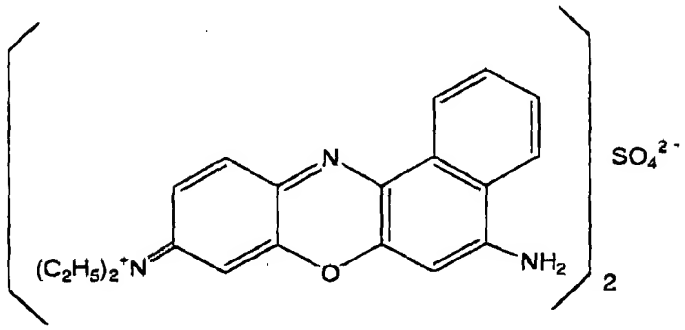
-LDS730:



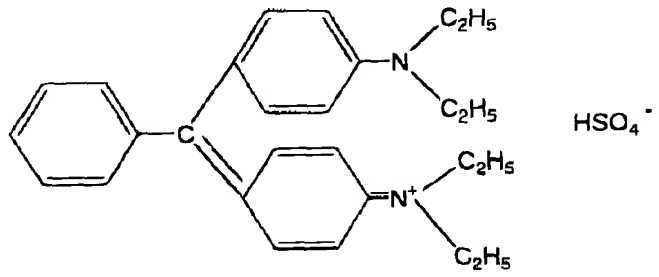
-LD700:



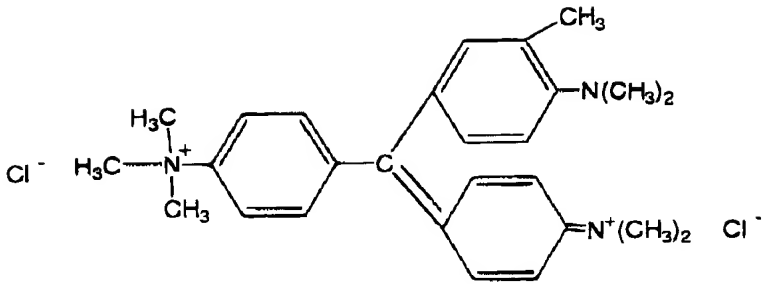
-Nile Blue A:



-Brilliant Green:

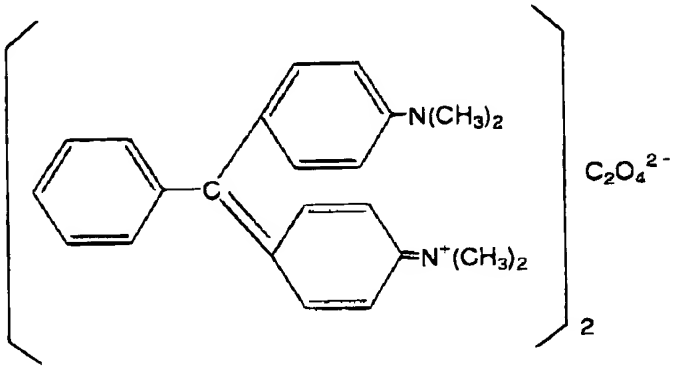


-Iodide green:



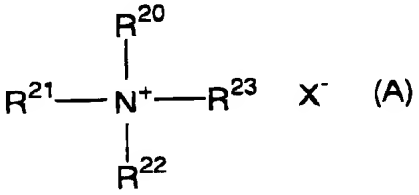
and

-Malachite green:



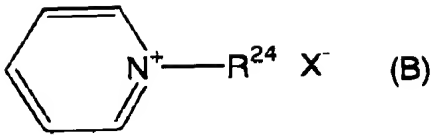
6. A method according to claim 1, wherein the erythrocyte lysing agent contains a surfactant, the surfactant comprises one or more surfactants selected from the group consisting of

- compounds of formula (A)



wherein, R^{20}, R^{21} and R^{22} are, the same or different, an hydrogen atom, a C_{1-8} alkyl group or a C_{6-8} aralkyl group; R^{23} is a C_{8-18} alkyl group, a C_{8-18} alkenyl group or a C_{6-18} aralkyl group; and X^- is an anion,

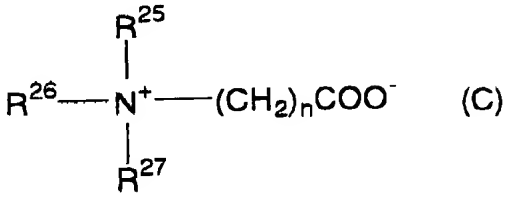
- compounds of formula (B)



(B)

wherein R^{24} is a C_{8-18} alkyl group; and X^- is an anion,

- compounds of formula (C)



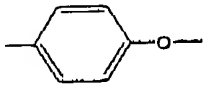
(C)

wherein R^{25} and R^{26} are, the same or different, a hydrogen atom, a C_{1-8} alkyl group, or a C_{6-8} aralkyl group; R^{27} is a C_{8-18} alkyl group, a C_{8-18} alkenyl group or a C_{6-18} aralkyl group; and n is 1 or 2,

- compounds of formula (D)

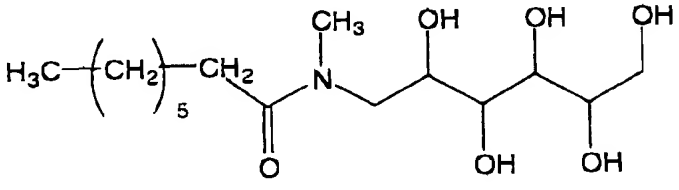
$$R^{28}-R^{29}-(CH_2CH_2O)_n-H \qquad (D)$$

wherein R^{28} is a C_{9-25} alkyl group, a C_{9-25} alkenyl group or a C_{9-25} alkynyl group; R^{29} is

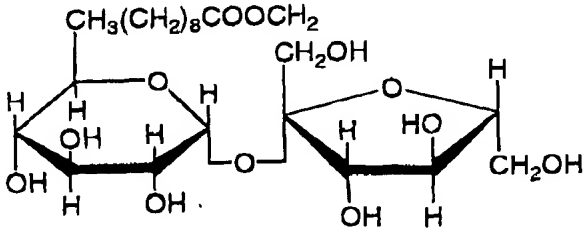


or $-COO-$; and n is an integer of 10 to 40,

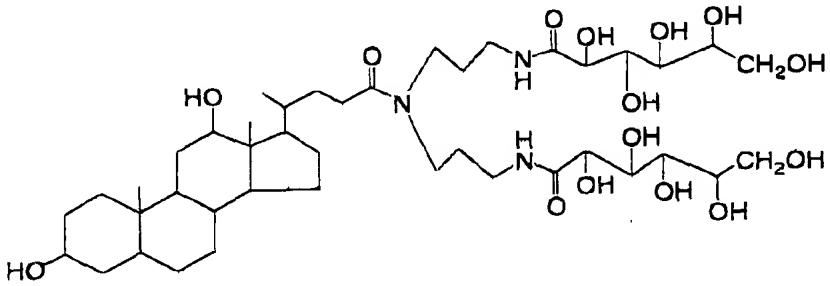
-MEGA-8:



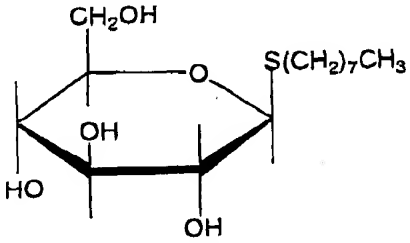
-sucrose monocaproate:



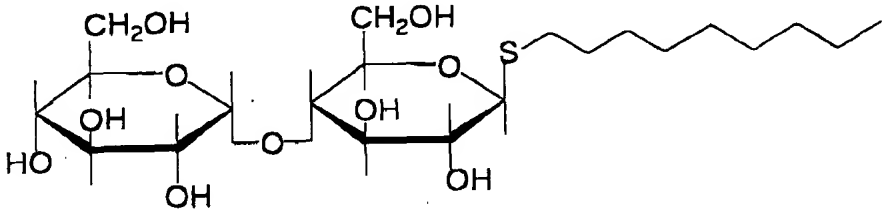
-Deoxy-BIGCHAP:



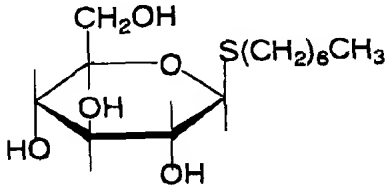
-n-octyl-β-D-thioglucoside:



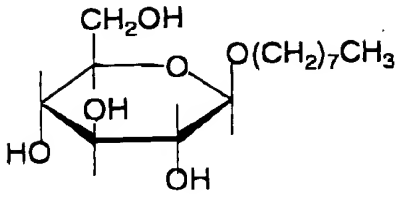
-n-nonyl-β-D-thiomaltoside:



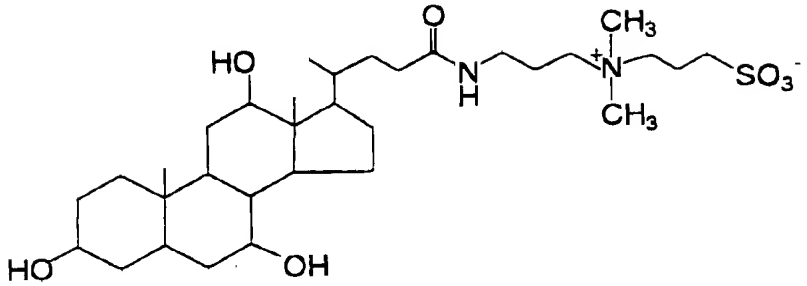
-n-heptyl-β-D-thioglucoside:



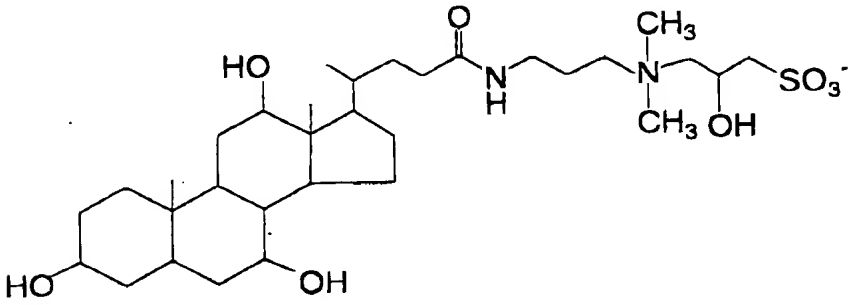
-n-octyl-D-oxyglucoside:



-CHAPS:



-CHAPSO:



5

7. The method according to claim 6, wherein the concentration of the surfactant is 10 to 10000 mg/L.

10 8. The method according to claim 1, wherein the detected scattered light is one or more kinds of scattered light selected from the group consisting of forward low-angle scattered light, forward high-angle scattered light and side scattered light.

Add B3